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Short communication

Diurnal variation in pilocarpine-induced generalized tonic–clonic seizure activity

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Abstract

The efficacy of the cholinergic agonist pilocarpine to evoke generalized seizures in rats was examined over the 24-h photocycle. Both seizure latency and severity during the 24-h period exhibited marked diurnal variation. Seizure activity generalized more rapidly throughout the dark phase and was often characterized by wild running clonus and tonic extensor convulsions. In contrast, electrographic seizures during the light phase were mainly associated with facial/forelimb clonus and rearing typical of pilocarpine-induced seizures. These data suggest that the sensitivity of the rodent brain to pilocarpine exhibits day–night variation and seizure activity induced during the dark phase may generalize via the brainstem. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Temporal clustering of spontaneous seizure activity is characteristic of both human and experimental epilepsy. Although the distribution of seizures tends to vary with the type of epileptic syndrome (Quigg, 2000), it is well documented that both chronic experimental seizures in rats and clinical seizures show daily periodicity and are more frequent during periods of inactivity or sleep (Shouse et al., 1996; Hellier and Dudek, 1999; Quigg et al., 2000). Since rats normally exhibit a rest–activity cycle that is out of phase with that of humans, spontaneous seizures in rats are common during the light phase of the photocycle. Additional evidence from experimental models has indicated that seizure thresholds in response to convulsive treatments also vary with the time of day. For example, rats display greater

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sensitivity to kainic acid- (Roberts and Keith, 1994), pentylenetetrazol- (Yehuda and Mostofsky, 1993), and 3-Mercaptoproprionic acid-induced (Golombek et al., 1992) convulsions during the dark than light phase. Although the physiological mechanisms are poorly understood, day–night variation in seizure susceptibility has been attributed to alterations in tissue responsiveness secondary to biochemical changes that occur throughout the photoperiod (Roberts and Keith, 1994).

In the present study, we examined day–night variation in convulsive activity of the cholinergic agonist pilocarpine (PILO). When administered to rats, PILO induces a period of status epilepticus that triggers pathophysiological changes in the forebrain such as neuronal cell loss and axonal sprouting, leading to increased excitation and the development of spontaneous recurrent seizures (Mello et al., 1993). For these reasons, the PILO model has been widely used to study various aspects of human temporal lobe epilepsy. In the PILO model, spontaneous recurring seizures tend to cluster during daylight hours (Arida et al., 1999). However, the relationship between photoperiod and sensitivity to the induction of cholinergic seizures has not been investigated.

2. Materials and methods

Male Wistar strain rats (Charles River, Canada) weighing 200–225 g were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and positioned in a stereotaxic frame. Extracellular electrodes were implanted bilaterally in stratum radiatum of hippocampal CA1 (from Bregma: P 3.5, L 2.7, V 3.3). The hippocampus is a key component of the forebrain seizure network and is consistently activated during PILO seizures (Leite et al., 1990; Mello et al., 1993; Arida et al., 1999). The electrodes (A-M Systems, Inc.) were 125 μm in diameter and Teflon-coated except at the cut tips. A recording ground was placed over the cerebellum. The assembly was fixed with acrylic cement to stainless steel screws inserted into the skull surface. Experiments began 1 week after surgery.

Under a 12:12 LD cycle (lights on at 08:00 h), a combination of scopalamine methylnitrate (1 mg/kg, i.p.) followed 30 min later by pilocarpine hydrochloride was administered systemically (380 mg/kg, i.p.) at one of eight fixed time points during the 24-h photocycle. Experiments conducted during the dark phase were performed under red light. For behavioral observations, a modified, 10-stage version of the Racine scale (Racine, 1972) was used to quantify seizure severity. Seizure onset latency was measured as the elapsed time from drug injection to the occurrence of a stage 4 (or higher) behavioral seizure (bilateral forelimb clonus with rearing). The upper end of the scale was defined as follows: stage 5, bilateral forelimb clonus with loss of posture; stage 6, multiple stage 5 seizures; stage 7, jumping; stage 8, running and jumping; stage 9, stage 8 followed by tonic seizure; stage 10, multiple stage 9 seizures (see Michalakis et al., 1998). For experiments conducted at 02:00 and 14:00 h, extracellular (EEG) recordings were made from the CA1 following pilocarpine injection. The EEG signal was amplified by a Grass Model 8-10 polygraph (filtered between 1 and 70 Hz) and sampled at 100 Hz using a Model 2800 digital oscilloscope (A-M Systems, Inc.).

At the conclusion of the experiment, rats were anesthetized with urethane and transcardially perfused with 0.9% saline followed by 10% formalin. The brain was removed and cut into 40 μm frozen sections that were stained with thionin to identify electrode sites.

3. Results

The diurnal variation in PILO seizure onset latencies is shown in Fig. 1. An analysis of variance indicated a significant effect of time of drug injection on seizure latency [$F_{7,99} = 7.59$, $P < 0.01$]. Behavioral seizures occurred more rapidly during the 22:00–02:00 h interval than during the remaining time points. There was also a significant effect of time of drug injection on seizure severity [$F_{7,99} = 6.05$, $P < 0.01$].

During the light phase, motor seizures began with mild body tremor and salivation that pro-

gressed to bouts bilateral forelimb clonus with rearing (stage 4) and loss of posture (stage 5). A brief (< 5 s) bout of running behavior (stage 8) was observed for three cases in the 18:00 h group prior to the development forelimb clonus (stage 3). Seizure-induced behaviors during the light phase were similar to those previously reported (Leite et al., 1990). The normal EEG was in the 6–8 Hz range and showed amplitudes of < 1 mV (Fig. 2A) Clonic seizures were associated with high-amplitude fast activity admixed with periodic slow waves (Fig. 2C).

During the mid-dark phase (24:00–02:20 h), baseline hippocampal EEG showed similar frequency and amplitude to that during the light phase (Fig. 2B). However, seizures progressed to severe motor convulsions characterized by a sequence of behavior beginning with wild running/bouncing (stage 8), followed by tonus (i.e. limbs contracted and pressed against body), and tonic extension of the forelimbs and/or hindlimbs (stage 9). This behavioral pattern was consistently followed by a period of electrographic and behavioral depression (i.e. loss of righting reflex and immobility). Subjects that regained righting reflex frequently displayed a secondary progression to forelimb clonus within 10–20 min. The onset of wild running was preceded and accompanied by paroxysmal electrical activity in CA1 (Fig. 2D) that persisted during tonus and hindlimb extension. The latter forelimb clonus during otherwise behavioral arrest was characterized by epileptiform discharges in CA1 interrupted by periods of low-amplitude activity (Fig. 2E).

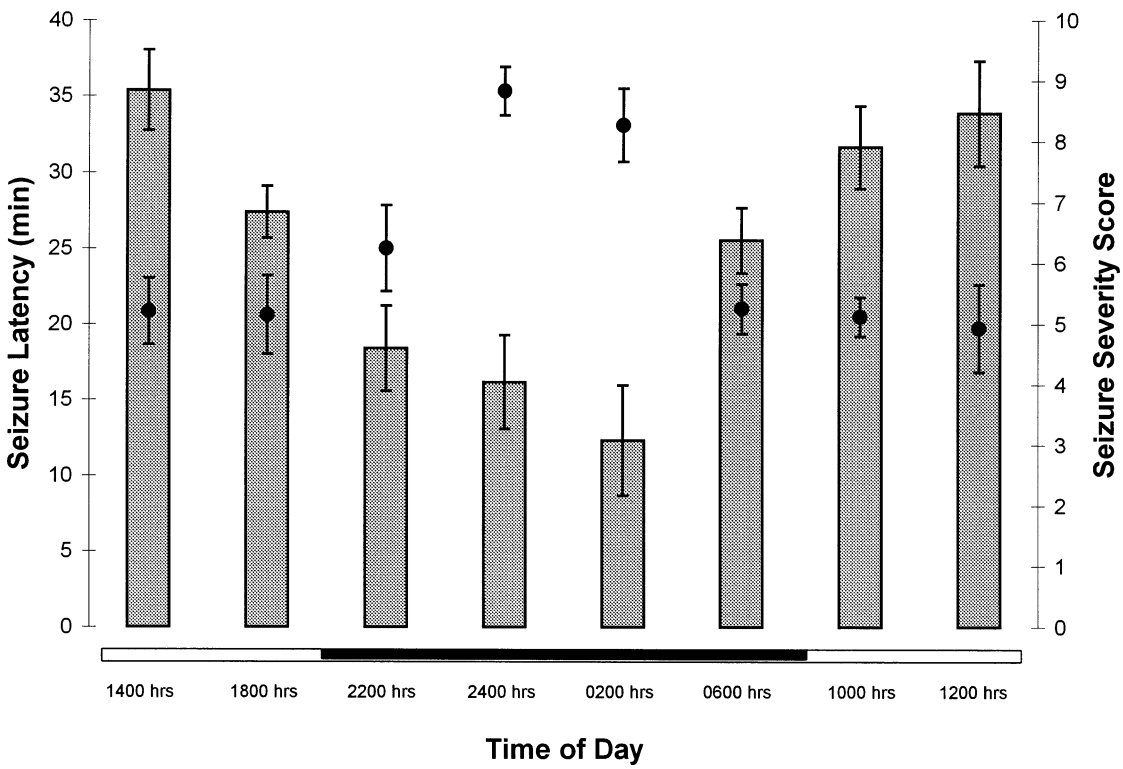

Fig. 1. Mean ($\pm$ S.E.M.) seizure latency (vertical bars) and severity (filled circles) following systemic PILO injection (380 mg/kg, i.p.) showed a diurnal rhythm ($N = 100$). Onset latency was defined as the time from PILO injection to the appearance of a stage 4 (bilateral forelimb clonus with rearing) or higher behavioral seizure. Seizure severity was ranked according to the scale of Michalakis et al. (1998). Note the decrease in seizure latency accompanied by an increase in seizure severity under dark conditions (i.e. 24:00 and 02:00 h).

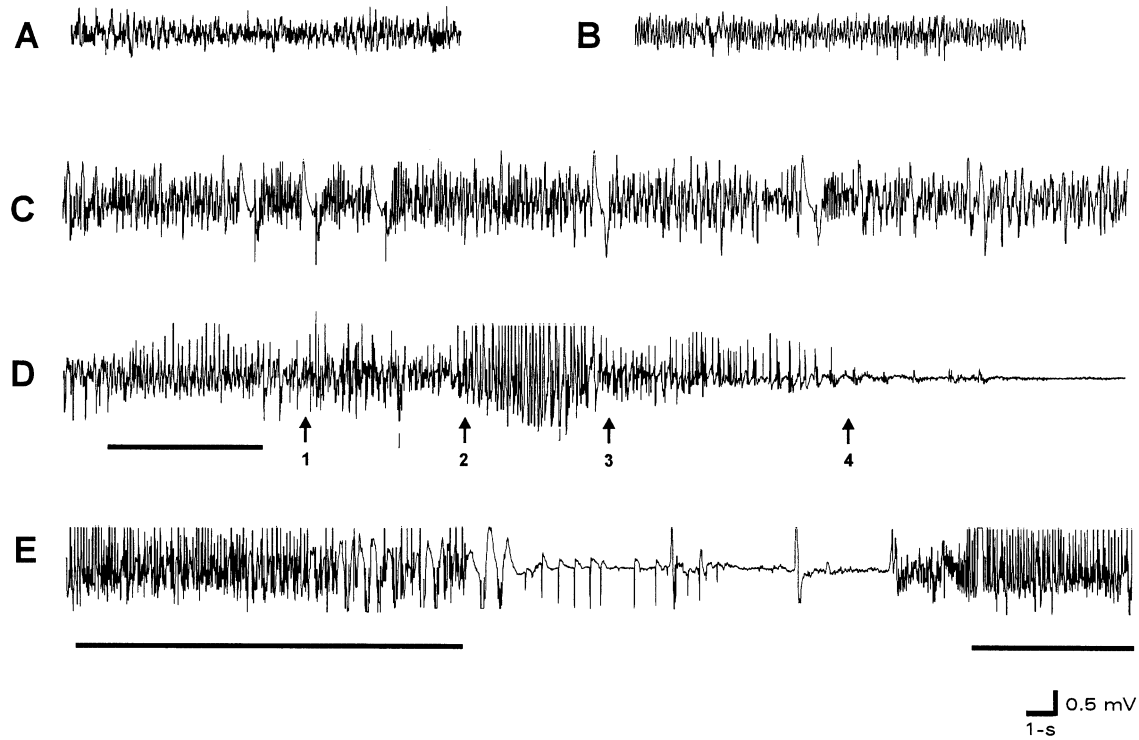

Fig. 2. Morphology of EEG recorded in the stratum radiatum of hippocampal CA1 during PILO seizures. Baseline EEG recorded 30 min prior to PILO injection in a representative rat at (A) 14:00 h ($N = 10$) and (B) 02:00 h ($N = 8$) was characterized by 6–8 Hz rhythmical slow activity and showed amplitudes of < 1 mV during periods of exploratory activity. (C) Represents the pattern of hippocampal EEG after onset of forelimb clonic seizures during light phase (14:00 h). (D) Shows the progression of electrographic activation at the different behavioral stages that accompanied onset of seizures induced during the dark phase (02:00 h): epileptiform activity preceding onset of wild running (horizontal bar), wild running (Arrow 1), tonus (Arrow 2), tonic hindlimb/forelimb extension (Arrow 3), and loss of righting reflex during electrographic and behavioral depresssion (Arrow 4). (E) The behavioral depression in (D) was followed by periods of high amplitude activity (horizontal bars) during bouts of post-tonic clonus (approximately 10–20 min after recovery of righting reflex).

4. Discussion

These data support and extend previous findings that the efficacy of chemoconvulsants varies with the time of day. The most significant result of this study however, is that seizure phenotype in the PILO model also shows day–night variation. During daylight hours, most rats exhibited forelimb clonic seizures with rearing and falling. This pattern of behavior is typical of PILO seizures observed during the daytime (Leite et al., 1990). Conversely, the same dose of PILO was shown to produce running clonus and tonic convulsions when administered during the dark phase. Light–dark variation in seizure phenotype has not been reported in the PILO model, although severe tonic convulsions have been observed with higher doses (i.e. 400–500 mg/kg) presumably during the day (Fujikawa, 1996).

The motor components of generalized seizures emerge from two distinct anatomical pathways: (1) a forebrain network implicated in facial and forelimb clonus (Michalakis et al., 1998), and (2) a brainstem network responsible for running/bouncing clonus and tonic convulsions (Hirsch et al., 1997; Faingold, 1999). The inferior colliculus (IC) plays a critical role in the initiation of brainstem seizures in a strain of genetically epilepsy-prone rats (GEPRs). In GEPRs, seizure discharges originate in the IC, then propagate to

deep layers of the superior colliculus (running phase), periaqueductal grey, and pontine reticular formation (tonic phase) (Faingold, 1999). Chemical or electrical stimulation of the IC in normal rats does not normally evoke tonic hindlimb extension unless brain norepinephrine (NE) levels are depleted (Browning et al., 1991; Bagri et al., 1999). The enhanced seizure susceptibility in GEPRs appears to partially result from a developmental deficiency in locus coeruleus (LC) NE projections innervating the superior colliculus (Ryu et al., 1999). Moreover, severe brainstem seizures have been reported to occur in response to stage 5 kindled forebrain seizures in GEPRs but not nonepileptic controls (Coffey et al., 1996), and it has been suggested that NE depletion in GEPRs is a contributing factor in the rostral to caudal spread of seizures (Proctor and Gale, 1997). Ascending NE projections from the LC innervate widespread areas of the forebrain and brainstem. NE depletion has been shown to reduce seizure threshold not only in GEPRs but also in a variety of experimental models and humans (Gale, 1992).

Noradrenergic modulation of seizure spread may explain the occurrence of brainstem-like seizures during the dark phase. It may be suggested that PILO seizures initiate in the forebrain and then spread to recruit a brainstem seizure network depending on the degree of NE activity within epileptogenic circuits. In normal rats, NE content in the LC exhibits daily variation and is low throughout most of the dark phase (Agren et al., 1986). Thus, a nocturnal decline in brain NE may have resulted in an increase in seizure susceptibility and allowed for forebrain seizure activity to propagate to and generalize via the brainstem.

Clarification of the gating mechanisms that modulate brainstem seizure susceptibility in the PILO model may provide insight into the evolution of tonic–clonic seizures in human partial epilepsy. Therapeutic interventions that target seizure-gating pathways could suppress secondary generalization and provide effective treatment against multiple types of convulsive seizures.

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References

Agren, H., Koulu, M., Saavedra, J.M., Potter, W.Z., Linnoila, M., 1986. Circadian covariation of norepinephrine and serotonin in the locus coeruleus and dorsal raphe nucleus in the rat. Brain Res. 397 (2), 353–358.

Arida, R.M., Scorza, F.A., de Araujo Peres, C., Cavalheiro, E.A., 1999. The course of untreated seizures in the pilocarpine model of epilepsy. Epilepsy Res. 34, 99–107.

Bagri, A., Di Scala, G., Sandner, G., 1999. Myoclonic and tonic seizures elicited by microinjection of cholinergic drugs into the inferior colliculus. Therapie 54 (5), 589–594.

Browning, R.A., Wang, C., Faingold, C.L., 1991. Effect of norepinephrine depletion on audiogenic-like seizures elicited by microinfusion of an excitant amino acid into the inferior colliculus of normal rats. Exp. Neurol. 112, 200–205.

Coffey, L.L., reith, M.E.A., Chen, N-H., Mishra, P.K., Jobe, P.C., 1996. Amygdala kindling of forebrain seizures seizures and the occurrence of brainstem seizures in genetically epilepsy-prone rats. Epilepsia 37 (2), 188–197.

Faingold, C., 1999. Neuronal networks in the genetically epilepsy-prone rat. Adv. Neurol. 79, 311–321.

Fujikawa, D.G., 1996. The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. Brain Res. 725, 11–22.

Gale, K., 1992. Subcortical structures and pathways involved in convulsive seizure generation. J. Clin. Neurophysiol. 9 (2), 264–277.

Golombek, D.A., Duque, D.F., De Brito Sanchez, M.G., Burin, L., Cardinali, D.P., 1992. Time-dependent anticonvulant activity of melatonin in hamsters. Eur. J. Pharmacol. 210, 253–258.

Hellier, J.L., Dudek, F.E., 1999. Spontaneous motor seizures of rats with kainate-induced epilepsy: effect of time of day and activity state. Epilepsy Research 35 (1), 47–57.

Hirsch, E., Danober, L., Simler, S., Pereira De Vasconcelos, A., Maton, B., Nehlig, A., Marescaux, C., Vergnes, M., 1997. The amygdala is critical for seizure propagation from brainstem to forebrain. Neuroscience 77 (4), 975–984.

Leite, J.P., Bortolotto, Z.A., Cavalheiro, E.A., 1990. Spontaneous recurrent seizures in rats: an experimental model of partial epilepsy. Neurosci. Biobehav. Rev. 14, 511–517.

Mello, L.E.A.M., Cavalheiro, E.A., Tan, A.M., Kupfer, W.R., Pretorius, J.K., Babb, T.L., Finch, T.L., 1993. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy. Epilepsia 34 (6), 985–995.

Michalakis, M., Holsinger, D., Ikeda-Douglas, C., Cammisuli, S., Ferbinteanu, J., DeSouza, S., Fecteau, J., Racine, R.J., Milgram, N.W., 1998. Development of spontaneous seizures over extended electrical kindling I. Electrographic, behavioral, and transfer kindling correlates. Brain Res. 793, 197–211.

Proctor, M., Gale, K., 1997. Basal ganglia and brainstem anatomy and physiology. In: Engel, J., Pedley, T.A. (Eds.), Epilepsy: A Comprehensive Textbook. Lippincott-Raven Publishers, Philadelphia, pp. 353–366.

Quigg, M., 2000. Circadian rhythms: interactions with seizures and epilepsy. Epilepsy Res. 42, 43–55.

Quigg, M., Clayburn, H., Straume, M., Menaker, M., Bertram, E.H., 2000. Effects of circadian regulation and rest-activity state on spontaneous seizures in a rat model of limbic epilepsy. Epilepsia 41 (5), 502–509.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. 32, 281–294.

Roberts, A.J., Keith, L.D., 1994. Sensitivity of the circadian rhythm of kainic acid-induced convulsion susceptibility to manipulations of corticosterone levels and mineralocorticoid receptor binding. Neuropharmacology 33 (9), 1087–1093.

Ryu, J.R., Jobe, P.C., Milbrandt, J.C., Mishra, P.K., Clough, R.W., Browning, R.A., Dailey, J.W., Seo, D.O., Ho, K.H., 1999. Morphological deficits in noradrenergic neurons in GEPR-9s stem from abnormalities in both the locus coeruleus and its target tissues. Exp. Neurol. 156, 84–91.

Shouse, M., Martins da Silva, A., Sammaritano, M., 1996. Circadian rhythms, sleep and epilepsy. J. Clin. Neurophysiol. 13, 32–50.

Yehuda, S., Mostofsky, D.I., 1993. Circadian effects of β-endorphin, melatonin, DSIP, and amphetamine on pentylenetetrazol-induced seizures. Peptides 14, 203–205.